Summary of Safety and Effectiveness Data

I. General Information

Device Generic Name: Assay hybridization and/or nucleic acid

Device Trade Name: Bayer VERSANT® HCV RNA 3.0 Assay (bDNA)

Applicant Name and Address: Bayer HealthCare LLC
Division Business Group Diagnostics
Street P.O. Box 2466
Berkeley, CA 94702-0466

Premarket Approval Application (PMA) Number: P020022

Date of Panel Recommendation: None

Date of Notice of Approval to Applicant: March 28, 2003

II. Indications for Use

The VERSANT® HCV RNA 3.0 Assay (bDNA) is a signal amplification nucleic acid probe assay for the quantitation of human hepatitis C viral RNA (HCV RNA) in the serum or plasma (EDTA and ACD) of HCV-infected individuals using the Bayer System 340 bDNA Analyzer. Specimens containing HCV genotypes 1-6 have been validated for quantitation in the assay.

The VERSANT HCV RNA 3.0 Assay (bDNA) is intended for use as an aid in the management of HCV-infected patients undergoing anti-viral therapy. The assay measures HCV RNA levels at baseline and during treatment and is useful in predicting non-sustained virological response to HCV therapy. The results from the VERSANT HCV RNA 3.0 Assay (bDNA) must be interpreted within the context of all relevant clinical and laboratory findings.

Assay performance characteristics have been established only for individuals treated with interferon alfa-2b plus ribavirin. No information is available on the assay's predictive values when other therapies are used.

III. Contraindications: None

IV. Warnings and Precautions

Warnings and precautions for users of the Bayer VERSANT HCV RNA 3.0 Assay (bDNA) are stated in the product labeling.

V. Device Description

Kit Configuration and Components

For detection of HCV RNA, the VERSANT HCV RNA Assay (bDNA) contains the following major components:

Component	Description
Lysis Reagent	proteinase K solution with sodium azide (< 0.1%) and other preservatives
Capture Probes	synthetic oligonucleotides in buffered solution with sodium azide (< 0.1%) and other preservatives
Target Probes	synthetic oligonucleotides in buffered solution with sodium azide (< 0.1%) and other preservatives
Capture Wells	polystyrene microwells coated with synthetic oligonucleotides
Pre-Amplifier Probes	synthetic oligonucleotides in buffered solution with sodium azide (< 0.1%) and other preservatives
Amplifier Probe	synthetic oligonucleotides in buffered solution with sodium azide (< 0.1%) and other preservatives
Substrate	chemiluminescent substrate (Lumi-Phos® Plus)
Label Probe	enzyme-labeled synthetic oligonucleotides in buffered solution with sodium azide (< 0.1%) and other preservatives
High Positive Control	human plasma containing recombinant single-stranded phage DNA with sodium azide (< 0.1%) and other preservatives
Low Positive Control	human plasma containing BPL-treated HCV with sodium azide (< 0.1%) and other preservatives
Negative Control	human plasma nonreactive for HCV with sodium azide (< 0.1%) and other preservatives
Standards A, D	human plasma containing recombinant single stranded phage DNA with sodium azide (< 0.1%) and other preservatives
Standards B, C	human plasma containing recombinant single stranded phage DNA with sodium azide (< 0.1%) and other preservatives
Standard E	human plasma containing recombinant single stranded phage DNA with sodium azide (< 0.1%) and other preservatives

In addition, the following component is required:

Bayer System 340 bDNA Analyzer with Data Management Software (DMS), version 6.11.1 or greater, for the VERSANT HCV RNA 3.0 Assay (bDNA)

VI. Principle of Device Methodology

The VERSANT HCV RNA 3.0 Assay (bDNA) is a sandwich nucleic acid hybridization procedure for the quantitation of hepatitis C viral (HCV) RNA in human serum and plasma. After HCV genomic RNA is released from the virions, the RNA is captured to a microwell by a set of specific, synthetic oligonucleotide capture probes. A set of target probes hybridizes to both the viral RNA and pre-amplifier probes, which form the backbone of the bDNA complex. The capture probes and the target probes bind to the 5' untranslated and core regions of the HCV genome. Amplifier probes subsequently hybridize to the pre-amplifier probe, forming a branched DNA (bDNA) complex.

Multiple copies of an alkaline phosphatase (AP) labeled probe then hybridize to the immobilized bDNA complex. Detection is achieved by incubating the AP-bound complex with a chemiluminescent substrate. Light emission is proportional to the amount of HCV RNA present in each sample, and results are recorded in relative light units (RLUs) by the analyzer. A standard curve is prepared using light emission from standards containing known concentrations of recombinant single-stranded bacteriophage DNA. Concentrations of HCV RNA in specimens are determined from the standard curve.

VII. Alternate Practices and Procedures

Prediction of response or nonresponse to HCV antiviral therapy has been based on the qualitative detection of HCV RNA during and at the end of anti-viral therapy. There has not been an adequate method commercially available to predict nonresponse to antiviral therapy during the course of treatment. In addition, the infecting HCV genotype, individual's gender, previous therapy for HCV infection has been used as predictors of response or nonresponse to therapy.

VIII. Potential Adverse Effects of the Device on Health

Since the Bayer VERSANT HCV RNA 3.0 Assay (bDNA) is for *in vitro* diagnostic use, there is no direct adverse effect on the health of the patient. However, failure of the product to perform as indicated or human error in use of the product may lead to a false result.

In the event of an erroneous prediction of nonresponse to HCV antiviral therapy, there is a possibility that a patient would have anti-HCV treatment terminated or changed when unnecessary.

IX. Marketing History

The VERSANT HCV RNA 3.0 Assay (bDNA) is currently approved in several countries, including France, Canada, Australia, Switzerland and Portugal. The product has not been withdrawn from the market in any country for any reason relating to the safety and effectiveness of the device.

X. Summary of Non Clinical Studies

Instrumentation

Software and hardware verification testing was performed for the Bayer System 340 bDNA Analyzer with Data Management Software (DMS) for the VERSANT HCV RNA 3.0 Assay (bDNA). Appropriate information and study results were furnished demonstrating that the Bayer System hardware and software, used with the VERSANT HCV RNA 3.0 Assay (bDNA), functioned as described and had appropriate safeguards.

Device Stability

The stability of the VERSANT HCV RNA 3.0 Assay (bDNA) was assessed on three manufactured device lots. Evaluations of stability include testing the kits at periodic intervals through 14 months for all 3 lots. Throughout the study, assay results were compared to established specification ranges to evaluate potential assay deterioration. Results of these real time stability studies indicate that the VERSANT HCV RNA 3.0 Assay (bDNA) is stable for at least 12 months when stored at its labeled storage conditions of 2 to 8 °C (Box 1) and -60 to -80 °C (Box 2). (Note: Reagent components are shipped in two boxes. Box 1 contains reagent components that require storage between 2 and 8 °C, whereas the Box 2 reagent components require storage at -60 to – 80 °C. See the devices labeling for a breakdown of the various components contained in Box 1 and Box 2.)

Assay Standardization

The VERSANT HCV RNA 3.0 Assay (bDNA) is anchored to a reference standard (RNA transcript), which is evaluated by three independent, validated analytical methods (absorbance at 260nm, hyperchromicity and phosphate analysis). The quantity of RNA in the transcript (in copies/mL) is determined from its phosphate content. The phosphate assay utilizes dilutions of the United States National Institute of Standards and Technology (NIST) phosphate standard reference material and is, therefore, directly traceable to the US NIST.

Multiple kit lots were used to determine the conversion factor between the VERSANT HCV RNA Assay (bDNA) HCV RNA copies/mL and the World Health Organization's (WHO) HCV RNA International Units (IU). Dilutions of the WHO International Standard for hepatitis C virus (HCV) RNA[1] were tested in this evaluation. The conversion factor for changing HCV RNA copies/mL to WHO IU was determined to be 5.2 HCV RNA copies/IU.

Assay Quantitation Limits

In the scientific literature it has is reported that the VERSANT HCV RNA has a LoD of 3,200 HCV RNA copies/mL. Bayer furnished information showing that at the level of 3,200 HCV RNA copies/mL at least 95% (lower bounds 95% confidence interval) of specimens not containing HCV RNA were designated as no HCV RNA present. However, only 39.5% of specimens containing HCV RNA were reported as HCV RNA present. It was determined that a more appropriate LoD would be 5,200 HCV RNA copies/mL. At this level at least 95% (lower bounds of 95% confidence interval) of specimens contain HCV RNA were detected. The value of 3,200 HCV RNA copies/mL was redesignated the detection limit (DC) denoting that below this level there is a high probability that HCV RNA would not be detected. The following was added to the device's labeling to educate users on the difference between DC and LoD.

- The Limit of Detection (LoD) is the lowest concentration of virus that yields an assay result at or above the Detection Cutoff in 95% of replicate determinations. The LoD for this assay is 1,000 IU/mL (5,200 HCV RNA copies/mL).

- The Detection Cutoff (DC) is the point on the assay quantitation scale such that 95% of negative specimens produce results below this cutoff with 95% confidence. The DC for this assay is 615 IU/mL (3,200 HCV RNA copies/mL).

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[1] Hepatitis C virus RNA, 1st International Standard, 1997, NIBSC code 96/790

- The Upper Quantitation Limit (UQL) for this assay has been determined to be 7,690,000 IU/mL (40,000,000 HCV RNA copies/mL).

- The quantitation range of the assay is from 615 IU/mL (3,200 HCV RNA copies/mL) to 7,690,000 IU/mL (40,000,000 HCV RNA copies/mL).

Assay Linearity

The assay was shown to be linear from 615 to 7,690,000 IU/ml (3,200 to 40,000,000 HCV RNA copies/mL). In addition, assay linearity was confirmed using serial dilutions of patient specimens that spanned the range from approximately 481 to 4,800,000 IU/ml (2,500 to 25,000,000 HCV RNA copies/mL) that were collected as serum and in EDTA and ACD anticoagulants. The following graph is representative of the assay linearity shown, figure 2.

Figure 2. Linearity of the VERSANT HCV RNA 3.0 Assay (bDNA)
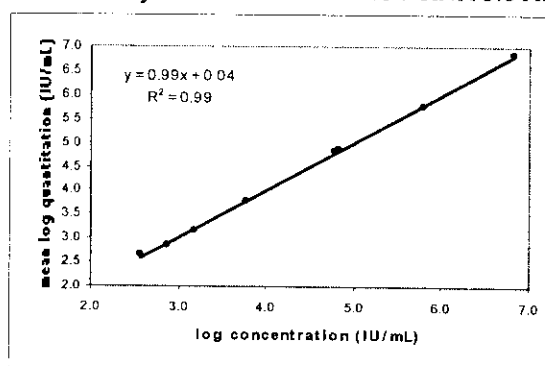
Comparison of Fresh Serum/Plasma Samples

Specimens were collected in serum (plastic serum separator tubes –SST-on and off the separator gel), EDTA (plastic K_2EDTA Vacutainer® tubes and plastic K_2EDTA PPT tubes with plasma stored on the gel), and ACD (glass ACD, Solution A) from 45 anti-HCV RNA nonreactive blood donors who were antibody negative for HIV, HCV, and HBsAg and from 25 HCV RNA reactive individuals with recent viral load assay results of > 962 IU/mL (5,000 HCV RNA copies/mL). RLUs and HCV RNA quantitations of HCV RNA nonreactive and HCV reactive serum specimens were compared to the matched anticoagulated plasma specimens. In order to establish equivalence between the test and reference conditions, the upper 95% confidence limits for the RLU ratios for HCV RNA nonreactive specimens had to be < 1.2 and the 90% confidence limits for the quantitation ratios for HCV RNA reactive specimens had to fall within the interval [0.70, 1.43].

The results of the study showed that there was minimal matrix effect when the anticoagulants were compared to serum (reference matrix), see table below for results containing HCV RNA.

Comparison of Fresh Serum/Plasma Samples

Matrix	Geomean Quantitation (HCV RNA copies/mL)	Ratio Compared to Reference	Lower 90% Confidence Limit of Ratio	Upper 90% Confidence Limit of Ratio
Serum (Reference)	4,634,164	--	--	--
Serum (On Gel)	4,718,033	1.02	0.94	1.10
K_2 EDTA Vacutainer	5,210,881	1.12	1.04	1.22
K_2 EDTA PPT (On Gel)	5,383,630	1.16	1.07	1.26
ACD	4,415,869	0.95	0.88	1.03

Slightly lower results were obtained with the specimens in ACD. This may be due to the dilutional effect of liquid anticoagulants. It is not believed that this effect will have a detrimental effect on the final assay results if all testing from an individual is performed with specimens collected in ACD. Essentially no differences were noted for the HCV RNA negative specimens tested. The above information shows that the assay should furnish relevant results for the matrices listed.

Comparison of Stability of Serum/Plasma Samples

Specimens prepared in the same manner as section 8.6 were used to evaluate the recommended storage conditions on final assay results. The reference condition was considered to be centrifugation, removal of plasma or serum, and freezing at -60 to -80 °C within 4 hours of collection except for the PPT tubes which were centrifuged within 2 hours. This was compared to conditions in which the serum and plasma specimens were held at 2 to 8 °C for 8, 24, or 48 hours before freezing at -60 to -80 °C, see table below for results obtained with samples containing HCV RNA.

Comparison of Stability of Serum/Plasma Samples

Matrix	Storage Time	N	Geomean[2] Quantitation (HCV RNA copies/mL)	Ratio Compared to Reference
Serum (Off Gel)	0 (Reference time)	25	4,634,164	----
	8	25	4,626,440	1.00
	24	25	4,712,195	1.02
	48	25	4,651,777	1.00
EDTA	0 (Ref)	25	5,210,881	
	8	25	5,286,557	1.01
	24	25	5,287,621	1.01
	48	25	5,357,185	1.03
ACD	0 (Ref)	25	4,415,869	
	8	25	4,387,779	0.99
	24	25	4,382,524	0.99
	48	25	4,418,249	1.00
SST (On Gel)	0 (Ref)	25	4,718,033	
	8	25	4,703,233	1.00
	24	25	4,654,383	0.99
	48	25	4,812,202	1.02
PPT (On Gel)	0 (Ref)	25	5,383,630	
	8	25	5,186,217	0.96
	24	25	5,110,336	0.95
	48	25	5,348,198	0.99

It appears that all sample matrices may be stored in accordance with Bayer's recommendations. Similar results were obtained with HCV RNA negative specimens.

Evaluation of Assay Reproducibility and Linearity across Matrices

Linearity, precision, and sensitivity across matrices were assessed using a six-member panel created from pooled specimens collected in serum, K_2EDTA, and ACD from two HCV RNA-positive individuals. The pooled undiluted specimen was pre-tested using the VERSANT HCV bDNA Assay to determine its concentration. The following table shows the reproducibility, expressed as percent correlation coefficient, of the assay across matrices:

Reproducibility

Reproducibility Matrix:Anti-Coagulant	Level 1 (neat)	Level 2 (2 x 10^6 HCV RNA copies/mL)	Level 3 (2 x 10^4 HCV RNA copies/mL)	Level 4 (10^4 HCV RNA copies/mL)	Level 5 (5 x 10^3 HCV RNA copies/mL)	Level 6 (2.5 x 10^3 HCV RNA copies/mL)
Serum	13.1%	11.6%	22.0%	24.9%	34.5%	40.0%
EDTA	14.4%	11.3%	22.7%	25.3%	29.3%	47.4%
ACD	12.7%	12.3%	27.4%	27.2%	34.6%	41.1%

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[2] Geomean = The geomean of n numbers is the nth root of the product, e.g., geomean for two numbers a1 and a2 is $\sqrt{a1 * a2}$, for three numbers a1, a2, a3 it is $\sqrt[3]{a1*a2*a3}$, etc.

Linearity was evaluated based on log differences from the expected concentration. Log differences for all matrices were within ± 0.1 log.

The above studies demonstrated that the VERSANT HCV RNA Assay has reasonable reproducibility and linearity across the matrices claimed.

Potentially Cross-Reacting and Interfering Substances

Cross-reacting and interfering substances were studied in HCV RNA-positive and HCV RNA-negative specimens to determine if these substances had an effect on assay specificity or HCV quantitation. The various substances or conditions were evaluated in 25 HCV RNA-positive and 35 to 45 HCV RNA-negative specimens, depending on the number of conditions tested. The following table shows all substances and conditions tested and their results:

Effect of Potentially Interfering Substances or Conditions on Quantitation of HCV RNA

Interfering Substance/Condition	Levels tested	Effect
Hemoglobin (upper limit of normal range 0.5 mg/dL)	500 mg/dL	No effect
Conjugated Bilirubin (upper limit of normal range = 0.3 mg/dL)	40 mg/dL	No effect
Unconjugated Bilirubin (upper limit of normal range = 1.1 mg/dL)	10, 20 and 40 mg/dL	Unconjugated bilirubin at 20 and 40 mg/dL had decreased quantitation in HCV RNA-positive specimens
Triglycerides (upper limit of normal range = 250 mg/dL)	3000 mg/dL	No effect
Protein (upper limit of normal is ~8 g/dL)	<8, 8-9, 9-10, 10-11 g/dL	Protein at > 9 g/dL had decreased quantitation in HCV RNA-positive specimens
Liver Enzymes (ALT, AST, GGT)	ALT 2x UNL; AST and GGT > UNL	No effect
Cryoprecipitates	Precipitate present, not quantified	No effect
Drugs*	5 X peak therapeutic concentration except as noted in footnote	No effect
Disease Pathogens**	10^5 CFU for bacteria and yeast. Concentrations of CMV, HIV-1, HAV, EBV, and HBV noted in footnote	No effect

*   Drugs Tested = Prednisone, ganciclovir, indinavir, lamivudine (tested at 1/2 C_{max}), Pegylated interferon alpha 2b (PEG-INTRON), cyclosporin, acyclovir, AZT, ritonavir, Ribavirin, alpha interferon (Intron A), tacrolimus, DDI, nelfinavir, D4T, Mycophenolate mofetil, rapamycin, azathioprine, saquinavir, amantadine, trimethoprim/sulfamethoxazole

** Disease Pathogens Tested = E. coli, P. aeruginosa, K. pneumoniae, H. influenzae, E. cloacae, P. fluorescens, S. aureus, S. marcescens, S. pneumoniae, S. epidermidis, Streptococcus group B, C. albicans, HIV-1 (10^6 HIV-1 RNA copies/mL), HAV (10^6 $TCID_{50}$), CMV (7×10^5 DNA copies/mL) and HBV (10^6 HBV DNA copies/mL)

For the substances and disease conditions tested there does not appear to be significant interference or cross-reactivity with the VERSANT HCV RNA Assay.

Genotype Detection

Two studies were performed to evaluate assay performance of HCV genotypes, 1, 2, 3, 4, 5, and 6 relative to genotype 1. The first study evaluated the ability of the VERSANT HCV bDNA Assay to quantitate non-1 genotypes. The second study evaluated the precision, linearity and analytical sensitivity of the VERSANT HCV bDNA Assay when measuring non-1 genotypes.

In the first study, RNA transcripts were prepared from the 5' untranslated region of cloned examples of HCV 1, 2, 3, 4, 5, and 6. Each transcript representing an HCV genotype was diluted to produce HCV RNA quantitations at approximately 384,615 (high concentration) and 1,346 (low concentration) IU/mL (2,000,000 and 7,000 HCV RNA copies/mL) relative to the VERSANT HCV bDNA Assay.

The relative recovery was calculated for each non-1 genotype as the ratio of the percent recovery of the non-1 genotype divided by the percent recovery for 1.

All of the non-1 genotype transcripts had quantitations that were within 1.5-fold of the genotype 1 transcript and of each other.

In the second study, precision, linearity and analytical sensitivity were assessed using panels made with specimens from three individuals infected with genotypes 1a, 1b, 2a, 2b, 3a, 4a, and 6a and one individual infected with genotype 5a. Each genotype specimen[3] was serially diluted to target concentrations of 385,000, 1,900, 960, and 480 IU/mL (2,000,000; 10,000; 5,000; and 2,500 HCV RNA copies/mL) as determined in the VERSANT HCV bDNA Assay. The lower concentration levels were chosen to bracket the estimated limit of detection, lower quantitation limit, and detection cutoff of the VERSANT HCV RNA 3.0 Assay (bDNA). Evaluation of the total %CVs for all the genotypes and dilution levels above the detection cutoff (615 IU/mL) showed that the assay quantitates all genotypes with acceptable precision (< 40%).

The estimated LoDs of all of the genotypes tested were within 1.3-fold of genotype 1.

Since the VERSANT HCV RNA 3.0 Assay (bDNA) assay has been standardized against the WHO HCV RNA Standard, which is HCV genotype 1[4] this would account for the variance seen with the other HCV genotypes. It is considered that the variance between HCV subtypes will not be an effectiveness issue if the device is used in accordance with its indications for use.

Stability

The stability of the VERSANT HCV RNA 3.0 Assay (bDNA) was assessed on three manufactured kit lots. Evaluation of stability included testing the kits at periodic intervals through 14 months for all three lots. Throughout the study, assay results were compared to established specification ranges to evaluate potential assay deterioration. Results of these real-time stability studies indicate that the VERSANT HCV RNA 3.0 Assay (bDNA) is stable for at least 12 months when stored at its labeled storage conditions of 2 to 8 °C (Box 1) and -60 to -80 °C (Box 2).

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[3] One patient specimen containing genotype 4 had an HCV RNA concentration of 6,480 IU/mL and therefore could not be tested at the high target concentration of 385,000 IU/mL.

[4] Saldanha J, Lelie N, Heath A, et. al., Establishment of the First International Standard for Nucleic Acid Amplification Technology (NAT) Assays for HCV RNA, Vox Sang 1999;76:149–158

Studies were conducted to ensure the shipping container would maintain the proper transport temperatures for up to 24 hours. Bayer states their shipping plan requires that domestic shipments arrive within 24 hours of shipping. The conditions tested were "summer" and "winter" conditions when the device would be shipped by overnight shipment. The shortest period of time found for transport temperatures to remain within specification was with "summer conditions" for 2 to 8 °C. This shipment went out of specification a mean of 41.8 hours. There was an equilibrium time for the containers when they were initially packed. This equilibrium period caused temperatures on the 2 to 8 °C container side to drop below 2 °C, e.g., -12 to +3 °C. Bayer states that they have internal data showing the DNA probes may be frozen and thawed multiple times.

The information provided supports a device expiration time of 12 months and a shipment time of 24 hours.

Reproducibility and Linearity

Assay reproducibility was established using a panel with HCV target concentrations between 615 and 7,690,000 IU/mL (3,200 and 40,000,000 HCV RNA copies/mL). Two panel members, QC1 and QC3, were constructed using recombinant single-stranded bacteriophage DNA as target material, diluted in human plasma matrix. The remaining panel members were beta-propriolactone (BPL)-treated HCV-positive patient specimens spiked into HCV RNA-negative human plasma. Varying concentrations were constructed by dilution. Two operators at each of 3 external trial sites performed the testing, with each operator performing 4 assay runs with each of the 3 clinical reagent lots. Each operator used a different instrument, i.e., different serial number.

The following shows, for each panel member, the number of replicates, the concentration, total log standard deviation (SD), Within-plate percent correlation coefficient (%CV) and total %CV derived from the above study.

Table 5. Total Log SD, Within-Plate %CV, and Total %CV for the HCV RNA Reproducibility Panel Members

Panel Member	N	Concentration in IU/mL (copies/mL)	Total log SD	Within Plate %CV	Total %CV
QC1	216	6,820,720 (35,467,746)	0.07	13.9%	17.0%
QC2	216	607,756 (3,160,333)	0.07	9.1%	17.0%
DT[1]	NA	100,000 (520,000)	0.07	NA	17.0%
QC3	216	68,207 (354,677)	0.06	10.2%	15.0%
QC4	216	60,776 (316,033)	0.07	9.3%	16.4%
DT	NA	10,000 (52,000)	0.09	NA	21.0%
QC5	216	6,078 (31,603)	0.08	10.9%	18.8%
QC6	216	1,519 (7,901)	0.10	18.9%	24.2%
QC7	216	760 (3,950)	0.12	24.2%	28.2%
DC[2]	NA	615 (3,200)	0.14	26.9%	32.4%
QC8	216	380 (1,975)	0.18	33.1%	42.2%

[1] DT= Decision Threshold Data from this reproducibility study were combined with data from reproducibility studies across specimen matrices and genotypes to obtain an estimate of the assay precision profile. A quadratic mode was filled o the precision profile to determine total reproducibility across sites, lots, operators, matricies, and genotypes at the decision thresholds.

[2] NA = Not applicable

[3] For the Detection Cutoff (DC), the within-plate, total % CV and total log SD were obtained by interpolation.

The Limit of Detection (LoD), defined as the HCV RNA concentration that is detected in 95% of replicate determinations with respect to the detection cutoff (DC) of 615 IU/mL, was estimated to be 1,000 IU/mL (5,200 HCV RNA copies/mL).

The assay has a quantitation range of 615 to 7,690,000 IU/mL (3,200 HCV RNA copies/mL to 40,000,000 HCV RNA copies/mL). Results reported between the DC of 615 IU/mL and the LoD of 1,000 IU/mL must be interpreted with caution because the detection rate of the assay gradually decreases from 95% at 1,000 IU/mL to 39.5% at 615 IU/mL and the variability of the assay increases from 26.6% CV at 1,000 IU/mL to 32.4% CV at 615 IU/mL.

The assay is linear from 615 IU/mL to 7,690,000 IU/mL (3,200 to 40,000,000 HCV RNA copies/mL). The accuracy of the assay, defined as the percent recovery (ratio of observed mean quantitation to expected concentration), was from 92% to 108% across the quantitation range. In addition, the linearity of the assay was confirmed using serially diluted individual patient specimens collected as serum and either EDTA or ACD anticoagulants (anticoagulants claimed for use).
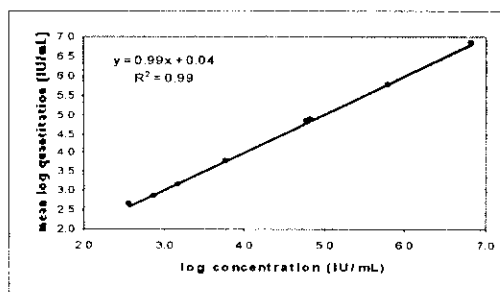
The above information demonstrates that the device has adequate reproducibility and linearity for the indication for use claimed.

Analytical Specificity for Limit of Detection and Detection Cutoff

Analytical specificity of the VERSANT HCV RNA 3.0 Assay (bDNA) was determined using 999 anti-HCV negative serum specimens from men and women of which the majority were repeat volunteer blood donors. The specimens were determined to be negative for antibodies to HCV using an FDA approved HCV ELISA assay. Samples were tested across four testing sites using three kit lots of the VERSANT HCV RNA 3.0 Assay (bDNA). Of the 999 samples tested, 992 (99.3%) produced results below the limit of detection (LoD) (<1,000 HCV RNA IU/mL or 5,200 HCV RNA copies/mL) with a lower 95% confidence limit of 98.7%. Alternatively, of the 999 samples tested, 987 (98.8%) produced results below the detection cutoff (<615 IU/mL or 3,200 HCV RNA copies/mL) with a lower 95% confidence limit of 98.1%.

Within Subject Variability

Since this assay is quantitative and will be used to test sequential specimens from the same individual it was necessary to establish within subject variability. Twenty-nine subjects (16 males and 13 females) with clinically stable, chronic HCV infection were enrolled in this study. Serum specimens were drawn from subjects weekly for 6 to 8 weeks with an average of 7 weeks. Enrollment and continued eligibility criteria for the study were: 1) No antiviral therapy or clinical events within the 3 months prior to the start of the study and 2) No initiation of antiviral therapy or clinical events during the 8-week study period. The ultimate objective of the study was to determine the minimum fold-change (ratio) between two successive measurements that is unlikely to occur by chance. The minimum fold-change was determined using the estimated total variability which is composed of assay and biologic variability. The fold-change for the VERSANT HCV RNA 3.0 Assay (bDNA) was estimated to be 2.6-fold or 0.41 logs. The biologic variability alone was 2.1 fold. The largest ratio between the minimum and maximum viral load within any subject was 1.6 logs and the median change across all subjects was 0.27 logs

XI. Summary of Clinical Studies

To determine the distribution of HCV RNA in chronically infected individuals, HCV RNA levels were measured by the Bayer VERSANT HCV RNA (bDNA) Assay in 650 chronically infected patients who were not undergoing therapy at the time of specimen collection. In 431 patients, quantification values were obtained from baseline specimens just before the initiation of anti-HCV therapy; the remaining 219 patients were not subsequently put on therapy. The figure below shows the distribution of HCV RNA log quantitations in HCV RNA IU/mL for the combined study population. The median quantitation was 5.8 log IU/mL with 5th and 95th percentiles of 4.5 log

IU/mL and 6.6 log HCV RNA IU/mL in the log scale, or a median of 694,566 HCV RNA IU/mL with 5th and 95th percentiles of 33,570 and 3,681,828 HCV RNA IU/mL.

Figure 1. Distribution of log HCV RNA in IU/mL (N = 650)

DC = Detection cutoff
UQL = Upper quantitation limit.
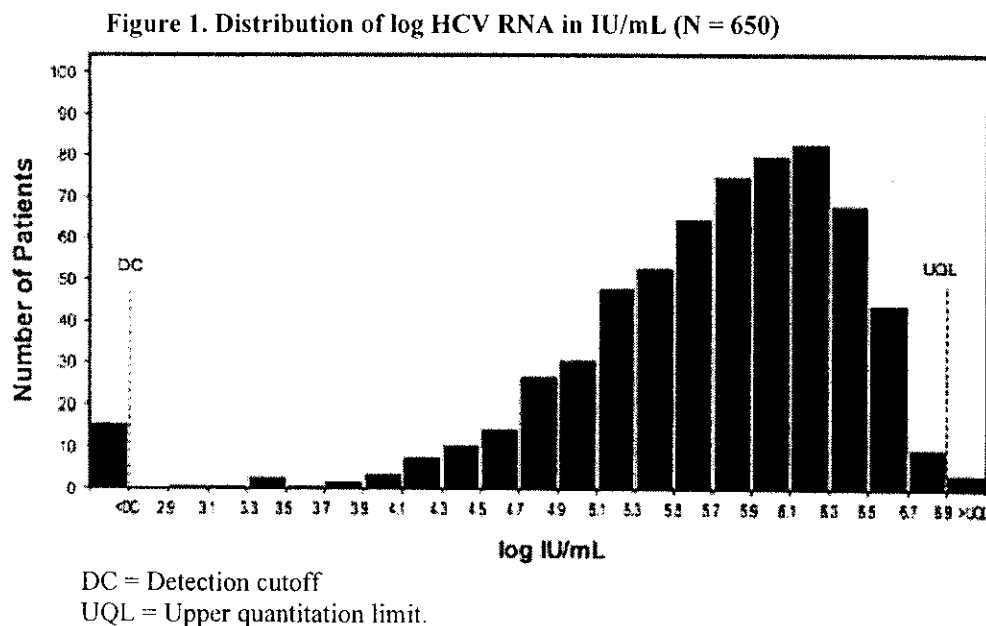
Bayer conducted a study in a group of individuals undergoing HCV antiviral therapy (interferon alfa-2b plus ribavirin – Rebetron®). The objectives of the study were to determine if HCV RNA quantitations or changes in quantitations were predictive of sustained virological response (SVR) and non-sustained virological response (NSVR) at post-treatment follow-up (PTF). Retrospectively collected specimens from 351 subjects treated with interferon alfa-2b plus ribavirin were assayed across six sites in North America (5) and Europe (1). Specimens were collected at baseline, weeks 4, 8, 12, 24 (for subjects who had 48 weeks of treatment), end of treatment (EOT), and post-treatment follow up (PTF). SVR was defined as having no detectable HCV RNA at PTF using a sensitive qualitative HCV RNA test. The population included previously treated and treatment-naive subjects. Table 4 shows the demographics for the Bayer study population.

Table 4. Description of Study Population

Characteristic	Number of Subjects (N = 351)	Percent of Subjects
Race		
Asian	14	4.0% (14/351)
African-American	4	1.1% (4/351)
Caucasian	235	67.0% (235/351)
Hispanic	16	4.6% (16/351)
Other[1]	82	23.4% (82/351)
Age Range		
20-24	2	0.6% (2/351)
25-34	29	8.3% (29/351)
35-44	135	38.5% (135/351)
45-54	130	37.0% (130/351)
55-59	28	8.0% (28/351)
60-64	16	4.6% (16/351)
65-74	11	3.1% (11/351)
Genotype[2]		
1	239	68.1% (239/351)
2	50	14.2% (50/351)
3	58	16.5% (58/351)
4	6	1.7% (6/351)
5	0	0.0% (0/0)
6	2	0.6% (2/351)
6-Month Treatment Duration	**162**	**46.1% (162/351)**
Genotype 1	85	52.5% (85/162)
Genotype non-1	77	47.5% (77/162)
1-Year Treatment Duration	**189**	**53.8% (189/351)**
Genotype 1	154	81.5% (154/189)
Genotype non-1	35	18.5% (35/189)
Previous Therapy	**76**	**21.6% (76/351)**
Males	54	71.0% (54/76)
Females	22	29.0% (22/76)
No Previous Therapy	**275**	**78.4% (275/351)**
Males	184	66.9% (184/275)
Females	91	33.1% (91/275)

1 Subjects treated and tested at the site in France did not have race recorded for legal reasons.

2 Percentages add up to greater than 100% because 4 subjects had mixed infections.

Table 5 shows the percent of sustained virological responders and odds ratios for prediction of SVR using baseline characteristics and treatment duration variables for the study population. The variables that were the most predictive of SVR (i.e., had the highest odds ratio) were genotype and baseline HCV RNA with a threshold of 100,000 (10^5) IU/mL (520,000 HCV RNA copies/mL).

Table 5. Percent Response and Odds Ratios for Prediction of SVR Using Baseline Characteristics[1]

Predictor	Variable	N	Percent with SVR	Odds Ratio (95% Confidence Interval)
Genotype	Non-1	112	70.5	4.3 (2.6, 7.1)
	1	239	36.0	
Baseline HCV RNA	$\leq 10^5$ IU/mL	63	66.7	2.7 (1.5, 5.0)
	$>10^5$ IU/mL	288	42.7	
Treatment Duration	48 Weeks	189	54.0	1.8 (1.2, 2.9)
	24 Weeks	162	38.9	
Liver Disease Stage[2]	NC	288	49.3	1.7 (0.9,3.1)
	C	63	36.5	
Age	<40	77	57.1	1.7 (1.0, 2.9)
	≥40	274	44.2	
Previous Treatment	Naive	275	48.7	1.4 (0.8, 2.4)
	Experienced	76	40.8	
Gender[3]	Female	113	49.6	1.2 (0.7, 1.9)
	Male	238	45.8	

1 Data shown are from the Bayer Clinical Study
2 NC = non-cirrhotic, C = cirrhotic
3 Early studies reported that gender was a statistically significant predictor of SVR. However, later studies have shown that gender and body weight are confounded and, when ribavirin dose is based on body weight, gender is no longer predictive of SVR.

For results obtained with the VERSANT HCV RNA 3.0 Assay (bDNA) in this study population, viral load decision thresholds ranging from 615 to 400,000 IU/mL and log drop in viral load (baseline to Week 4, 8, 12, or 24) thresholds ranging from 1 to 3 logs were evaluated for their ability to predict SVR and NSVR. Optimal viral load and log drop thresholds were selected at each time point based on the largest number of predicted NSVR and negative predictive values (NPV) ≥ 95%. Table 6 defines the final prediction rules selected.

Table 6. Definition of Prediction Rules.

Week	Type of Rule	Prediction Rule	Predicted Non-sustained Virological Responders	Predicted Sustained Virological Responders[1]
4	Viral Load Threshold	100,000 IU/mL[2]	≥ 100,000 IU/mL	< 100,000 IU/mL
	Log Drop[1]	1 Log Drop	≤ 1 log and ≥ 1,000 IU/mL[2]	> 1 log or < 1,000 IU/mL
8, 12 and 24	Viral Load Threshold	10,000 IU/mL[2]	≥ 10,000 IU/mL	< 10,000 IU/mL
	Log Drop	2 Log Drop	≤2 log and ≥1,000 IU/mL	> 2 log or < 1000 IU/mL

1 Log drops are calculated relative to pretreatment baseline viral load
2 100,000 IU/mL = 520,000 HCV RNA copies/mL, 10,000 IU/mL = 52,000 HCV RNA copies/mL, 1,000 IU/mL = 5,200 HCV RNA copies/mL

Table 7 shows the above rules applied to the study population, the NPV and positive predictive values (PPV) of the viral load and log drop rules for Weeks 4, 8, 12, and 24 (for subjects receiving 48 weeks of treatment) and the corresponding adjusted and unadjusted odds ratios for SVR. The adjusted odds ratios are based on multivariate logistic regression models. The final model selected adjusted for the following variables: genotype, liver disease stage, length of therapy, previous treatment, and age.

Table 7. Predictors of Sustained and Non-Sustained Virological Response at Weeks 4, 8, 12, and 24 and Corresponding Odds Ratios for SVR

Week	Prediction Rules	Negative Predictive Value[1]		Positive Predictive Value[2]		Odds Ratio[3]	
		NPV (95% CI)	N	PPV (95% CI)	N	Unadjusted	Adjusted
4	100,000 IU/mL	96.6 (88.3-99.6)	57/59	55.5 (49.2-61.7)	142/256	35.5	26.4
	1 Log Drop[4]	94.0 (86.7-98.0)	79/84	60.2 (53.5-66.5)	139/231	23.9	22.3
8	10,000 IU/mL	98.7 (92.8-100.0)	74/75	66.5 (59.6-73.0)	135/203	146.9	117.8
	2 Log Drop	97.5 (91.2-99.7)	77/79	67.3 (60.3-73.8)	134/199	79.4	69.1
12	10,000 IU/mL	97.1 (90.1-99.7)	68/70	59.1 (52.6-65.3)	143/242	49.1	29.8
	2 Log Drop	97.4 (90.9-99.7)	75/77	60.9 (54.3-67.1)	143/235	58.3	43.1
24[5]	10,000 IU/mL	100 (81.5-100)	18/18	64.5 (54.6-73.5)	69/107	NA	NA
	2 Log Drop	100 (82.4-100)	19/19	65.1 (55.2-74.1)	69/106	NA	NA

1 NPV = Percent of non-sustained virological responders out of the patients who are predicted non-sustained virological responders; i.e. HCV RNA positive 6 months post-therapy.

2 The positive predictive value (PPV) of HCV RNA levels is not intended to be used to guide therapy. PPV = Percent of sustained virological responders out of the patients who are predicted sustained virological responders; i.e. HCV RNA negative 6 months post-therapy.

3 Odds ratios cannot be calculated if the NPV is 100%. Those cases are indicated by NA. All odds ratios were significant (p <.001).

4 None of the log drop rules at Week 4 attained an NPV of 95%. The 1-log drop had the highest NPV.

5 For subjects receiving 48 weeks of treatment

To determine if there was a difference for the VERSANT HCV RNA 3.0 Assay (bDNA) to predict SVR or NSVR in individuals where the HCV genotype was known Bayer evaluated the study data broken out by HCV genotype (1 and non-1). Table 8 shows prediction for individuals infected with HCV genotype 1 for NSVR and SVR.

Table 8. HCV Genotype 1: Predictors of SVR and NSVR at Weeks 4, 8, 12, and 24

Week	Prediction Rules	Negative Predictive Value[1]		Positive Predictive Value[2]	
		NPV (95% CI)	N	PPV (95% CI)	N
4	100,000 IU/mL	96.5 (87.9-99.6)	55/57	46.6 (38.7, 54.6)	75/161
	1 Log Drop[3]	93.3 (85.1-97.8)	70/75	50.3 (41.9, 58.8)	72/143
8	10,000 IU/mL	98.5 (92.1-100.0)	67/68	57.6 (48.2, 66.7)	68/118
	2 Log Drop	97.2 (90.3-99.7)	70/72	58.8 (49.2, 67.9)	67/114
12	10,000 IU/mL	96.9 (89.3-99.6)	63/65	49.0 (40.9, 57.2)	75/153
	2 Log Drop	97.1 (90.1-99.7)	68/70	50.7 (42.3, 59.0)	75/148
24[4]	10,000 IU/mL	100 (81.5-100)	18/18	59.3 (48.2, 69.8)	51/86
	2 Log Drop	100 (82.4-100)	19/19	60.0 (48.8, 70.5)	51/85

1 NPV = Percent of non-sustained virological responders out of the patients who are predicted non-sustained virological responders; i.e. HCV RNA positive 6 months post-therapy.

2 The positive predictive value (PPV) of HCV RNA levels is not intended to be used to guide therapy. PPV = Percent of sustained virological responders out of the patients who are predicted sustained virological responders; i.e. HCV RNA negative 6 months post-therapy.

3 None of the log drop rules at Week 4 attained an NPV of 95%. The 1-log drop had the highest NPV.

4 For subjects receiving 48 weeks of treatment

Table 9 shows prediction for individuals infected with HCV genotype non-1 for NSVR and SVR.

Table 9. HCV Genotype Non-1: Predictors of SVR and NSVR at Weeks 4, 8, 12, and 24

Week	Prediction Rules	Negative Predictive Value[1]		Positive Predictive Value[2]	
		NPV (95% CI)	N	PPV (95% CI)	N
4	100,000 IU/mL	100 (15.8, 100)	2/2	70.5 (60.3, 79.4)	67/95
	1 Log Drop	100 (66.4, 100)	9/9	76.1 (65.9, 84.6)	67/88
8	10,000 IU/mL	100 (59.0, 100)	7/7	78.8 (68.6, 86.9)	67/85
	2 Log Drop	100 (59.0, 100)	7/7	78.8 (68.6, 86.9)	67/85
12	10,000 IU/mL	100 (47.8, 100)	5/5	76.4 (66.2, 84.8)	68/89
	2 Log Drop	100 (59.0, 100)	7/7	78.2 (68.0, 86.3)	68/87
24[3]	10,000 IU/mL	NA	NA	85.7 (63.7, 97.0)	18/21
	2 Log Drop	NA	NA	85.7 (63.7, 97.0)	18/21

1 NPV = Percent of non-sustained virological responders out of the patients who are predicted non-sustained virological responders; i.e. HCV RNA positive 6 months post-therapy.

2 The positive predictive value (PPV) of HCV RNA levels is not intended to be used to guide therapy. PPV = Percent of sustained virological responders out of the patients who are predicted sustained virological responders; i.e. HCV RNA negative 6 months post-therapy.

3 For subjects receiving 48 weeks of treatment.

The NPVs for genotype non-1 for both viral load and log-drop rules were 100% at Weeks 4, 8, and 12 but sample sizes for subjects with NSVR were small, ranging from 2 to 9 patients. It is believed that the lower sample sizes for subjects with NSVR reflect the fact that subjects infected with non-1 genotypes have a better response to therapy than subjects infected with genotype 1.

From the information above it can be seen that the NPVs of the viral load prediction rules were >95% at all four timepoints, independent of genotype. The highest NPV of the log drop in viral load rule was 93.3% at Week 4, using a 1-log drop. The NPVs for a 2-log drop at weeks 8, 12, and 24 were >95% at all three timepoints, independent of genotype. While the PPVs were lower than the NPVs, ranging on average from 55% to 67% for both viral load threshold and log drop, they were slightly higher than the expected percent of SVR of 47% at baseline than if other predictive factors, e.g., HCV genotype, baseline viral load, were taken into consideration.

The data from this study using a 2-log drop rule are consistent with those reported in the National Institutes of Health (NIH) Consensus Development Conference Statement, Management of Hepatitis C: 2002.[5] The NIH statement was based primarily on study populations treated with pegylated-interferon plus ribavirin.

Based on the clinical studies mentioned above Bayer established the following interpretation of result table showing probabilities for treatment failure.

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[5] National Institutes of Health Consensus, Management of Hepatitis C: 2002, June 10-12,2002; Preliminary Draft Statement, June 12, 2002; Final Statement, Revisions made September 12, 2002

Interpretation rules using absolute viral load:
- At week 4, if HCV RNA is $\geq$100,000 IU/mL, then there is a 97% chance of treatment failure.
- At week 8, if HCV RNA is $\geq$10,000 IU/mL, then there is a 99% chance of treatment failure.
- At week 12, if HCV RNA is $\geq$10,000 IU/mL, then there is a 97% chance of treatment failure.
- At week 24, if HCV RNA is $\geq$10,000 IU/mL, then there is a 100% chance of treatment failure.

Interpretation rules using decrease in viral load compared with pretreatment baseline level:
- At week 4, if HCV RNA is $\geq$10% ($\leq$1 log drop) of its pretreatment baseline level and $\geq$1,000 IU/mL, then there is a 94% chance of treatment failure.
- At week 8, if HCV RNA is $\geq$1% ($\leq$2 log drop) of its pretreatment baseline level and $\geq$1,000 IU/mL, then there is a 97% chance of treatment failure.
- At week 12, if HCV RNA is 1% ($\leq$2 log drop) of its pretreatment baseline level and $\square$1,000 IU/mL, then there is a 97% chance of treatment failure.
- At week 24, if HCV RNA is $\square$1% ($\leq$2 log drop) of its pretreatment baseline level and $\leq$1,000 IU/mL, then there is a 100% chance of treatment failure.

It is recommended that the above interpretations be included with the patient's report.

From the above information it appears that the Bayer VERSANT HCV RNA (bDNA) Assay should be safe and effective when used in the population indicated and according to its directions for use.

XII. Conclusions Drawn from Studies

The described preclinical and clinical studies demonstrated the following:

- The detection cutoff of 615 IU/mL (3,200 HCV RNA copies/mL) and a limit of detection of 1000 IU/mL (5,200 HCV RNA copies/mL) were confirmed, as shown in the Analytical Specificity and Reproducibility Studies

- The assay is linear and reproducible from 615 IU/mL to 7,690,000 IU/mL (3,200 c/mL to 40,000,000 copies/mL), which defines the quantitation range of the assay.

- The endogenous substances hemoglobin, conjugated bilirubin, triglycerides, elevated liver enzymes, and cryoprecipitates appear not to interfere with assay performance. Unconjugated bilirubin did not appear to interfere with assay results in HCV RNA-negative specimens, but levels >10 mg/dL resulted in lower quantitations in HCV RNA-positive specimens. A level of 10 mg/dL unconjugated bilirubin is well outside the upper limit of the normal range (~1.1 mg/dL) and it is believed would rarely be encountered. Protein levels above 9 g/dL were associated with decreased HCV quantitation in HCV-positive specimens.

- None of the therapeutic drugs tested interfered with assay performance, nor did viruses, bacteria, or yeast that were tested.

- Specimens undergoing up to 4 freeze-thaw cycles appeared to have no change in their final result.

- Serum and K_2 EDTA and ACD Solution A anticoagulants can be used for specimen collection for the VERSANT HCV bDNA Assay. Serum and plasma from EDTA and ACD Vacutainer tubes can be held at 2-8 °C for up to 48 hours before freezing at –60 to –80 °C.

- RNA transcripts from HCV genotypes 2, 3, 4, 5, and 6 quantitated within 1.5-fold of genotype 1. Dilutional panels prepared from specimens collected from patients with each of these different genotypes were found to have reproducibility, linearity, and analytical sensitivity similar to those of genotype 1.

Clinical Studies

- Study results indicated that virtually all subjects with chronic HCV infection have HCV RNA concentrations that are quantifiable in the VERSANT HCV bDNA Assay. Greater than 99% of the baseline specimens from subjects enrolled in HCV treatment had viral loads > 615 IU/mL (3,200 copies/mL), and 94% of a population of subjects with chronic HCV infection, not in treatment, had quantifiable levels of HCV RNA. There were no apparent differences in quantitations between males and females that would place limitations on the use of the assay.

- The specificity study results showed there were no false reactive results for the diseases tested.

- The within-subject variability data, when combined with estimates of variance from the reproducibility study, showed that a fold-change of 2.6 (0.41 log) or greater was unlikely to occur by chance and could be used as an indicator of a true change in HCV RNA viral load. The biologic variability alone was 2.1 fold.

- The clinical study showed that both baseline and during-treatment viral loads were statistically significant predictors of non-sustained viral response. At assessment weeks 4, 8, 12, and 24, during-treatment viral loads provided negative predictive values of > 95% for subjects who were not sustained virological responders. Log drops in viral loads also provided NPVs of > 95% at weeks 8, 12, and 24.

Safety

As a diagnostic test, the VERSANT HCV RNA 3.0 (bDNA) assay involves removal of blood from an individual for testing purposes. The test, therefore, presents no more safety hazard to an individual being tested than other tests where blood is removed.

Benefit/Risk

The submitted clinical studies demonstrate that the VERSANT HCV RNA 3.0 (bDNA) Assay, when compared to current methods for assessing response to anti-viral therapy, has a similar ability to predict nonresponse to antiviral for individuals infected with HCV. It has been shown that the device has no demonstrable cross-reactivity with viruses or organisms that may cause clinical hepatitis. Therefore, this device should benefit the physician in predicting antiviral response in individuals chronically infected with HCV.

The results of the preclinical and clinical laboratory studies the VERSANT RNA 3.0 (bDNA) Assay, provide reasonable assurance the device is safe and effective when used in accordance with the device labeling.

XIII. PANEL RECOMMENDATION

In accordance with the provisions of section 515(c)(2) of the act as amended by the Safe Medical Devices Act of 1990, this PMA was not referred to the Microbiology Advisory Panel, an FDA advisory committee, for review and recommendation because the information in the PMA substantially duplicates information previously reviewed by this panel.

XIV. CDRH Decision

FDA issued an approval order on March 28, 2003.

The applicant's manufacturing facility inspected on February 20, 2002 and was found to be in compliance with the Quality Systems Regulation (21 CFR 820).

XV. Approval Specifications

Directions for use: See Labeling

Hazards to Health from Use of the Device: See Indications, Contraindications, Warnings, precautions and Adverse Events in the labeling.

Postapproval Requirements and Restrictions: See approval order.